

Supplemental Material

Table S1. Oligonucleotides used for conventional PCR or real-time PCR.

Table S2. Sequences of shRNAs used, purchased from Sigma-Aldrich.

Figure S1. Sequencing data on the HIV integration site in J-Lat E27 and splicing events detected.

Figure S2. Activation of latent full-length HIV down-regulates host gene expression at the integration site.

J-Lat 6.3 and 8.4 cells were untreated or treated with 5-aza-2'-deoxycytidine (aza-dC; $5\mu\text{M}$) for 48 h and TNF- α (10ng/ml) for the last 24 h, RNA was extracted and transcription through the PPP5C gene was measured by RT-qPCR (right panel). GAPDH expression was measured for normalization. Data is expressed as fold change (FC) PPP5C/GAPDH expression in treated cells compared to untreated cells. The left panel shows HIV reactivation in J-Lat 6.3 and 8.4 cells upon treatment, expressed as % GFP-positive cells. Clone J-Lat 6.3 contains a sense latent integration of a full-length HIV virus (HIV-R7/E⁻/GFP) at intron 4 of the PPP5C gene (Serine/threonine-protein phosphatase 5; chromosome 19), and J-Lat 8.4 contains an antisense integration of HIV at the DNA helicase V gene [1,2].

Figure S3. HIV induction upon serum starvation affects UBXD8 expression.

J-Lat E27, A2 and H2 cells were grown in normal media containing 10% fetal bovine serum (+FBS) or in media without sera (-FBS) for 48 h, RNA was extracted and transcription through UBXD8 exon 8 was measured by RT-qPCR (right panel). GAPDH expression was measured for normalization. Data is expressed as fold change (FC) UBXD8/GAPDH expression in -FBS cells compared to +FBS cells. The left panel shows HIV reactivation in J-Lat E27 cells upon serum starvation expressed as % GFP-positive cells.

Figure S4. Spt6 depletion enhances reactivation in J-Lat cells containing a latent full-length HIV provirus.

J-Lat 8.4 cells infected with Control or Spt6 shRNA expression vectors and puromycin-selected for 6 days, were treated or not with aza-dC ($5\mu\text{M}$) for 48 h and TNF- α (10ng/ml) for the last 24 h. HIV-GFP expression was measured by FACS, and expressed as percentage of GFP-positive cells (%GFP) (right panel). Depletion of Spt6 was tested by RT-qPCR with specific primers and is shown in the left panel.

Figure S5. Analysis of HIV transcription in J-Lat E27 cells upon Chd1 depletion. J-Lat E27 cells depleted of Chd1 as in Figure 5 were RNA-extracted and HIV expression was measured by RT-qPCR with different amplicons covering the HIV genome as in Figure 5E.

Figure S6. Increased sensitivity of chromatin to MNase digestion upon PMA treatment. Resistance of chromatin to MNase in J-Lat E27 cells treated with PMA (10 nM) for 30 min, compared to untreated cells, was assayed and expressed as in Figure 6A.

Figure S7. Effect of Spt6 depletion on cellular gene expression in clone J-Lat A2.

RNA was extracted from J-Lat A2 cells expressing Control or Spt6 shRNA, and expression of the indicated exons of UBXD8 and UTX genes was measured by RT-qPCR using specific oligonucleotides. RPL31 expression was measured for normalization. In order to compare between different amplicons, qPCR was performed in parallel from genomic DNA (gDNA). Data is expressed as relative units (RU) [cDNA amplification/RPL31]/gDNA amplification.

Figure S8. The Hir complex and the histone chaperon Asf1 are required for the repressive role of the HIV R/U5 region on yeast basal transcription. A wild-type yeast strain and the indicated isogenic mutants were transformed with plasmids pTy1-HIV and pTy1-HIVTARless and the levels of HIV-derivative mRNAs were quantified by Northern hybridization. Deletion of *HIR1*, *HIR2*, *HIR3*, *HPC2* and *ASF1* eliminate the negative effect of the R/U5 region on the levels of a chimeric HIV-yeast mRNA, driven by the U3 region of the Ty1 retroelement under non-inducing conditions, without affecting the TAR-less control construct. A representative Northern hybridization, as well as the average of three independent experiments, is shown. Error bars indicate SD.

Figure S9. Effect of ASF1a, HIRA and Spt16 depletion on UBXD8 gene expression in clone J-Lat E27.

RNA was extracted from J-Lat E27 cells expressing Control or the indicated shRNA, and expression of UBXD8 was measured by RT-qPCR using specific oligonucleotides. As a control, expression of UBXD8 and CXCR4 was measured from J-Lat A2 expressing the same shRNAs. RPL31 expression was measured for normalization.

Figure S10. Occupancy of Sp1 on the latent HIV locus.

Chromatin obtained from J-Lat E27 cells treated or not with TNF- α (10ng/ml) for 8 h was immunoprecipitated with an antibody against Sp1 (sc59, Santa Cruz), and qPCR was performed with the primer pairs indicated. Results are presented as fold enrichment over a no-antibody control.

REFERENCES

1. **Jordan, A., D. Bisgrove, and E. Verdin.** 2003. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J.* **22:**1868-1877. doi: 10.1093/emboj/cdg188 [doi].
2. **Lenasi, T., X. Contreras, and B. M. Peterlin.** 2008. Transcriptional interference antagonizes proviral gene expression to promote HIV latency. *Cell. Host Microbe.* **4:**123-133. doi: 10.1016/j.chom.2008.05.016.

Table S1.

Name	Sequence of primers
1	CTTCGTTTATACGGCCTGA
2	TTGCGACAAAACTCATCAGA
3	AACAGCAAAAGTTGGCAGAG
4	GAAACTTTCTGGGCTTCC
5	AAATTAGCTAGGCATGGTGGTA
6	GCCTTGTGTGTTGGTAGATCC
7	CGAGCTTGCTACAAGGGACT
8	AGTAGTGTGTGCCGTCTGT
9	TCGCTTCAGGTCCCTGTCG
10	TTCAGCTATTCTAAATGCCACA
11	TTGGCGTACTCACCAAGTCGC
12	TTGTAGCACCATCCAAGGT
13	GAGCCCTCAGATCCTGCATA
14	CCTTCTTCTCGATTCCCTCG
15	CCACCTTCTTCTCGATTCCCT
16	TCTGAAGGGATGGTTGTAGC
19	GCTGAAGGATCGAAGGATGA
20	TCTGCCAACCTTGCTGTTG
21	TTCTAGGCGTTCTGACACCA

Name	Sequence of primers
Tat_f	CAAAAGCCTTAGGCATCTCCT
Tat_r	CCACCTTCTTCTCGATTCCCT
GFP_f	GAAGCAGCACGACTTCTCAA
GFP_r	GCTTGTGGCCATGATATAGA
17	ATCCACTGACCTTGGATGG
18	GTACTCCGGATGCAGCTCTC

Name	Sequence of primers
CHES1_f	GGCTTATGGCTTTGGAAACA
CHES1_r	ATCTGATGGTGCCAGGAAG
CXCR4_f	TGGAGTGTGACAGCTTGGAG
CXCR4_r	GGTGGTCTATGTTGGCGTCT
PCAF_f	ACATCTGCCATTCCCAACTC
PCAF_r	TAGCCATTGAGGGTTCTT
11beta_HSD2_f	ACGCAGGCCACAATGAAGTAG
11beta_HSD2_r	GCAGCCAGGCTGGATGATG
UBXD8_exon3_f	GCAAGAGGGCGTACCTAGTG
UBXD8_exon3_r	CTTGGTTGAGGTCTTGAGACAA
UBXD8_exon8_f	GCTGAAGGATCGAAGGATGA
UBXD8_exon8_r	TTCTAGGCGTTCTGACACCA
UBXD8_exon9_f	CAGACCCAAGTGCTGAGACA

UBXD8_exon9_r	TCTGCCAACTTTGCTGTTG
UTX_exon1_f	GGCGATAAAAGTTGGTGTGCT
UTX_exon1_r	GACACTCCGCAGGATTCAT
UTX_exon6_f	GGCAATTAAAGCATTAGGAG
UTX_exon6_r	TTTGAACATAAGCCAAGTCG
UTX_exon12_f	ATCAGCCCAGGATGCTTTA
UTX_exon12_r	GTGCTGCAAGTGCAGAGGTA
GAPDH_f	GAGTCAACGGATTTGGTCGT
GAPDH_r	TTGATTTGGAGGGATCTCG
RPL31_f	CGGCTGTCCAGAAAACGTA
RPL31_r	ACGTGTGTCACCCCTAGCTT
PPP5C_f	GAATCGAGAACGCCATCAAG
PPP5C_r	TGCGGTTGCCATAGTAGATG

Name	Sequence of primers
SPT6_f	CTGATTGAATTGCCAGGT
SPT6_r	GATCAAGGCCTGGCTGTAAG
CHD1_f	GATGAAGATTGGCAAATGTCTG
CHD1_r	ATTTGAGGTTTCTGCTTTG
ASF1A_f	ATGTGGCTCTGCAGAAAGT
ASF1A_r	TTAGGTGCATCAGCCTGAAA
HIRA_f	CACAGCTACTGCCACTGGAC
HIRA_r	ACGGGGTCGTTAACACAGAA
SUPT16H_f	GGCAAAATTGCCACTGTCTT
SUPT16H_r	GATATGGCCTCCCTCCTCT

Name	Sequence of primers
M1	AAGGGCTAATTCACTCCAAA
M2	GGCCCTGGTGTGTTCTG
M3	CACACAAGGCTACTCCCTGA
M4	AGCACCATCAAAGGTCAGT
M5	ATCCACTGACCTTGGATGG
M6	TGGCCTTTCTACCTTATCTGG
M7	GAGGCCAATAAAGGAGAGAAC
M8	ACACTCTCTCTCCGGGTCA
M9	ATGGATGACCCGGAGAGAG
M10	CACGTGATGAAATGCTAGGC
M11	TCTCTGGCTAACTAGGGAACCCA
M12	GCACTCAAGGCAAGCTTATTGA
M13	AAGCCTCAATAAGCTTGCCTTG
M14	AGTCACACAACAGACGGGCAC
M15	AGTGTGTGCCGTCTGTTGTG
M16	TGACTAAAAGGGCTGAGGGATCTA
M17	GAGATCCCTCAGACCCTTTAGTCA

M18	TTCAAGTCCCTGTTCGGGC
M19	GTGGCGCCCGAACAGG
M20	TTGCCGTGCGCGCTT
M21	CAGGACTCGGCTTGCTGAAG
M22	CACCAAGTCGCCGCC
M23	CGACTGGTGAGTACGCCAAA
M24	ATCTCTCCTCTAGCCTCCGC
M25	GCGGAGGGCTAGAAGGAGAGAGAT
M26	TAATACCGACGCTCTCGCACC
M27	GCGAGAGCGTCGGTATTAGC
M28	TTTCCCCCTGGCCTTAACC
M31	GGGAGCTAGAACGATTCGCAG
M32	CTGATGTCTAAAGGCCAGGATTA
M33	GATT CGCAGTTAAC CCTGGCCT
M34	CCAGTATTG TCTACAGCCTCTGATGT
M35	CATCAGAAGGCTGTAGACAAAT
M36	GGATGGTTTAGCTGTCCCAG
linker_f	TGACCCGGAGAGAGAAGTGT
linker_r	GTACTCCGGATGCAGCTCTC
M51_f	ACCTCTGGGGCTCTACCAAT
M51_r	CTAAAAGCTAATAGCGGCCTGA
M52_f	GAGTTCCCTCTGGAACAGAGA
M52_r	ATTGGTAGAGCCCCAGAGGT
M53_f	AGCGAGACCCATCTACA
M53_r	CAAGGACCTGGGACTACAGG

Table S2.

shRNA	Sigma code	Sequence	Exon
SPT6	TRCN0000019732	CGCCTTGTACTGTGAATTAT	23
CHD1	TRCN0000021312	GCAGTTGTGATGAAACAGAAT	5
ASF1A	TRCN0000074269	CCAAATCTACAGTCAC TTCTT	4
HIRA	TRCN0000145800	GCAGGC GATTCTGTCAATAAAA	14
SUPT16H	TRCN0000001258	CGCAAGTCTAATGTGT CCTAT	14
BRG1	TRCN0000015552	CGGCAGACACTGTGATCATT	25

Figure S1. Sequencing data on the HIV integration site in J-Lat E27 and splicing events.

A. Integration site of HIV at the UBXD8 gene in clone J-Lat E27

ACTAGTCTGAGCCTGGGAGCTCTCGGCTAACTAGGAAACCCACTGCTTAAGCCTCAA
TAAAGCTTGCCTTGAGTGTCAAGTAGTGTGTGCCGTCTGTTGTGACTCTGGTAA
CTAGAGATCCCTCAGACCCCTTGTAGTCAGTGTGGAAAATCTCTAGCAGCCAGGAGTTCG
AGGCAGCAGTGAAGCTCTGATTGTGCCTCTGCACCTCCAGCCTGGATGACAGAGCAGGACT
CTGTCTT

Red: 3'LTR ('R-U5) of HIV-1.

Black: intron 8 of gene UBXD8. The HIV integration divides intron 8 in two pieces of 404 bp (upstream) and 1846 bp (downstream).

B. Spliced transcripts

A₂ transcript (primers 1+12)

TTTATACGGCCTGACCCTCGCAGCCGGTCTCTGACCCGTTGGGACATTGTTTCATT
TAGGGGGTCTTGAAGAGAAATATGGGAGGGCACACCCGTCTTCTACCAGGGAACGT
ACAGCCAGGCACCTAACGATGCCAAAAGGGAGCTCGCTTCTTTGGTTATCTTCAT
GGAGATGATCACCAAGGACTCTGATGAGTTTGTGCAACACACTCTGTGCACCTGAAGT
TATTTCACTAATAAACACTAGGATGCTCTGGCATGCTCTACAAACAAACCTGAGG
GATACAGGGTCTCACAGGCTTACGAGAGAACACCTATCCATTCTGGCCATGATTATG
CTGAAGGATCGAAGGATGACTGTGGTGGACGGCTAGAAGGCCTCATTCAACCTGATGA
CCTCATTAACCAACTGACATTATCATGGATGCTAACAGACTTACCTGGTGTCAAGAAC
GCCTAGAAAGAACTACACACAGGCCAGGGTTCAGATTGCAT

Black: UBXD8 exon 5.

Green: UBXD8 exon 6.

Blue: UBXD8 exon 7.

Violet: UBXD8 exon 8.

Red: truncated 5'LTR (U3) of HIV-1 due to cryptic splicing acceptor.

C₂ transcript (primers 13+14)

CTGCTTTGCTTGTACTGGGTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTC
TGGCTAACTAGGAAACCCACTGCTTAAGCCTAATAAGCTTGCCTTGAGTGCTTCAAG
TAGTGTGTGCCGTCTGTTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTAG
TCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAA

CCAGAGGAGCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCAGA
GGGGCGGCGACTGGAAGAAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACT
CATCAAGTTCTATCAAAGCAACCCACCTCCAATCCGAGGGGACCCGACAGGCC
GAAGGAATCGAAGAAGAAGGAG

Red: 5'LTR ('U3-R-U5) of HIV-1.

Black: pre-gag sequence until splicing donor.

Violet: truncated Tat sequence due to cryptic splicing acceptor.

C₃ transcript (primers 13+14)

TACCCTGCTTTGCTTGTGACTGGGTCTCTGGTTAGACCaGATCTGAGCCTGGGAGCT
CTCTGGCTAACTAGGAAACCCACTGCTTAAGCCTCAATAAAGCTTGCTTGAGTGCTTC
AAGTAGTGTGTGCCGTCTGTTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTT
TAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCGAACAGGGACTTGAAAGCGAAAGGG
AAACCCAGAGGAGCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGG
CGAGGGCGGCACTGACCCACCTCCAACCCCGAGGGGACCCGACAGGCC

Red: 5'LTR ('U3-R-U5) of HIV-1.

Black: pre-gag sequence until splicing donor.

Violet: truncated Tat sequence due to cryptic splicing acceptor.

F transcript (primers 8+4)

TGTGCATCTGTACTGAGTCCCTGACCCCTTTAGTCAGTGTGGAAAATCTCTAGCAGTG
GCGCCCGAACAGGGACTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCGACGCAGGA
CTCGGCTTGCTGAAGCGCGCACGGCAAGAGGGCGAGGGGGCGCGACTGGAAAGAAAGAAA
CCAGACCCAAGTGCTGAGACAACAGCAGGATGAGGCCTACCTGGCTCTCAGAGCTG
ACCAGGAGAAAGAAAGAAAGAACGGGAGGAGCGCTAAGCGCGGAAGGGAGGAG
GAGGTGCAACAGCAAAAGTTGGCAGAGGAGAGACGGCGGCAGAATTACAGGAGGAAA
GGAAAGGAAGTTGGAATGCCTGCCCTGAACCTTCCCCTGATGACCCTGAAAGTGTCA
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TCTCTAAC

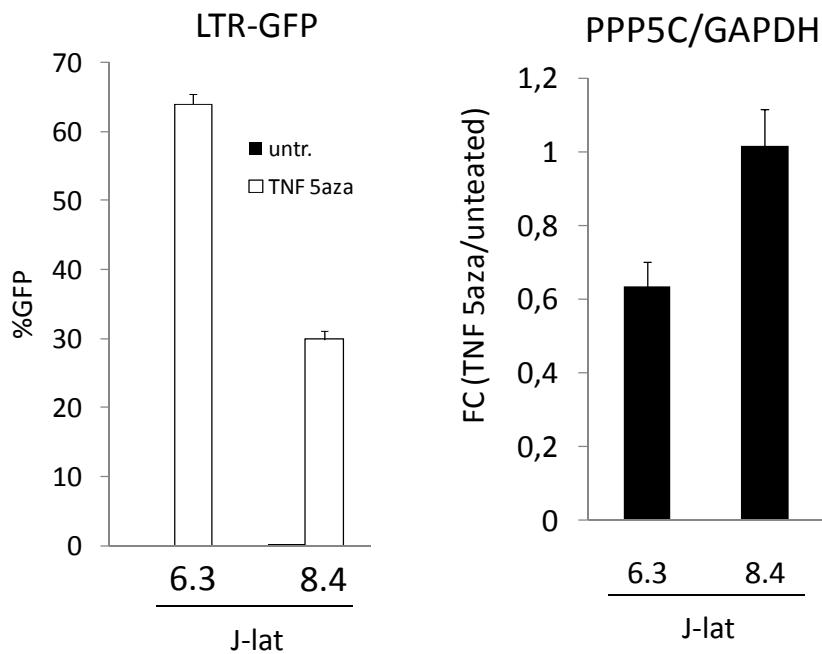
Red: 5'LTR ('U5) of HIV-1.

Black: pre-gag sequence until splicing donor.

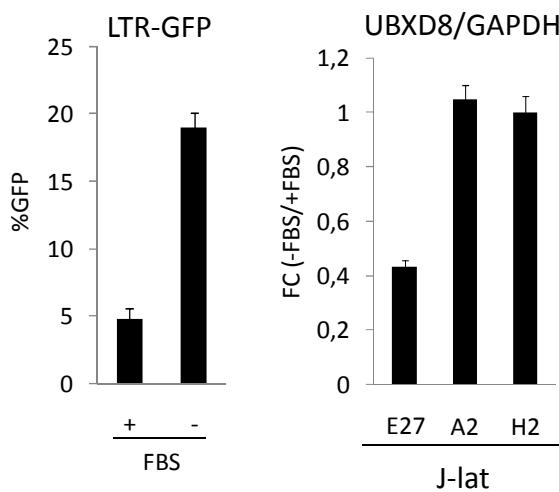
Blue: UBXD8 exon 9.

Violet: UBXD8 exon 10.

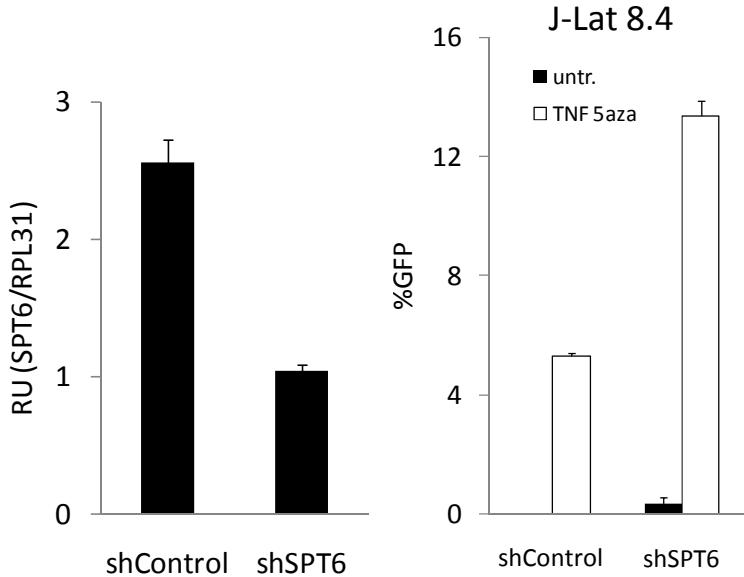
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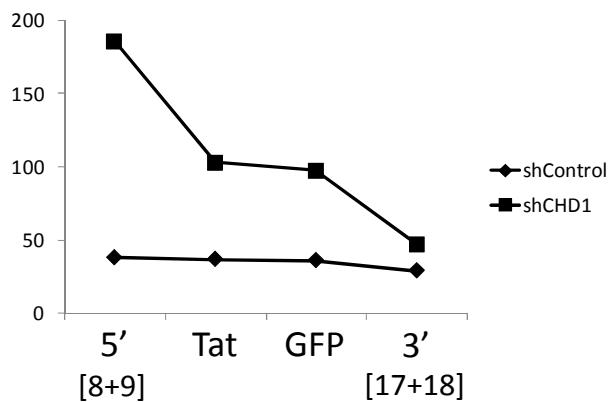
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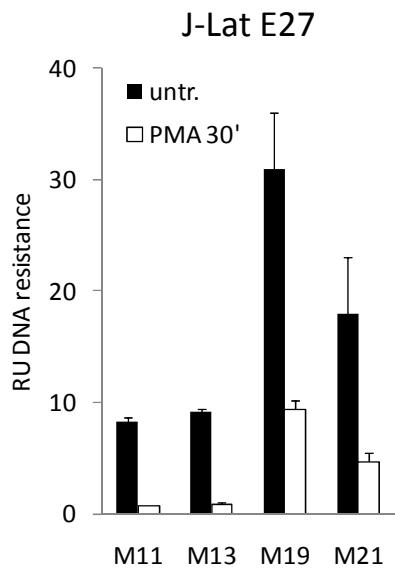
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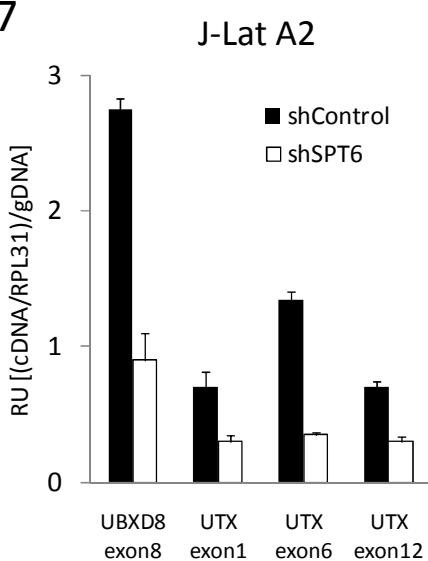
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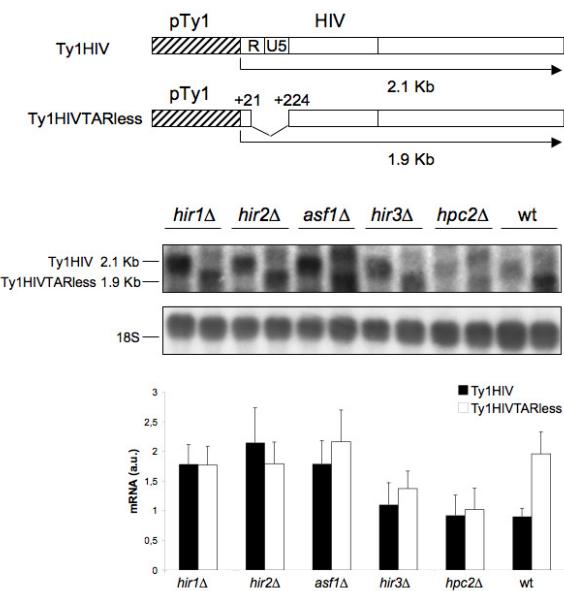
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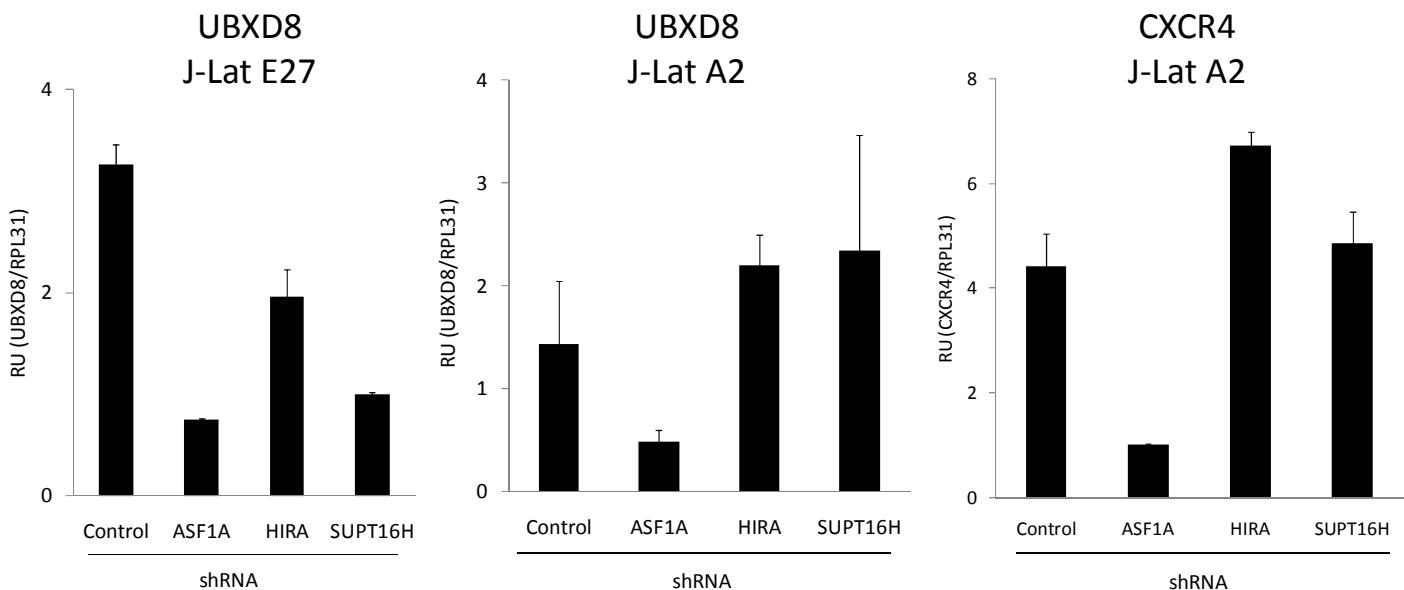
S7



S8



S9



S10

